Revised synopsis

on

Anti-psoriatic and Anti-inflammatory studies on Berberine Chloride loaded nanovesicles carrier

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DECLARATION

I hereby declare that the dissertation synopsis entitled "**Anti-psoriatic and anti-inflammatory studies on berberine Chloride loaded nanovesicles carrier**" to be submitted for the partial fulfillment of the Degree of Doctor of Philosophy is my original work and the dissertation has not formed the basis for the award of any degree, diploma, associateship or fellowship of similar other titles. It has not been submitted to any other University or Institution for the award of any degree or diploma.

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Introduction

Psoriasis may be defined as chronic inflammatory and immune-mediated disorder (Nimisha et al, 2020). It is characterized by well-defined plaques bearing adherent and silvery scales. There are several types of psoriasis including plaque (the most common type), guttate, inverse, erythrodermic, and pustular, including impetigo herpetiformis, that affects the skin, nails, and less frequently the joints (Bae et al, 2012). Inflammatory skin diseases are cause of important adverse events and disability for a large number of patients, thus negatively affecting their quality of life. The incidence of these skin diseases, which include atopic dermatitis and psoriasis acute, recurrent or chronic, is increasing in recent years, especially in developed countries (Villa et al, 2019). In psoriasis, the skin becomes rigid due to increase in the level of cholesterol and decrease in ceremides level which leads to a reduction of water content in the skin. This possesses a big challenge to make drug available to the target tissue using topical route (NImisha et al, 2020). During the last decades, the use of phytodrugs for the treatment of different skin disorders has aroused a great scientific interest. Like other natural antioxidants, it is in vivo biological effectiveness is limited by low bioavailability especially when applied on the skin. To overcome these limitations, the loading of natural antioxidants into nanocarriers has been proposed aiming at increasing their bioavailability and reducing possible side effects (M. Pleguezuelos-Villa et al, 2019).

Fig 1. Psoriasis

Different types of Nano carriers

Liposomes: Liposomes (phospholipid-based vesicles) have been investigated since 1970 as a system for the delivery or targeting of drugs to specific sites in the body (Gregory et al, 2012). Liposomes are small artificial vesicles of spherical shape that can be created from cholesterol and natural non-toxic phospholipids. Due to their size and hydrophobic and hydrophilic character (besides biocompatibility), liposomes are promising systems for drug delivery (Abolfazl et al, 2013). Liposomes have been proposed and considered as one of the most suitable topical drug delivery systems thanks to their ability to increase drug accumulation at the site where its activity is desired (Maria et al. 2017).

Glycerosomes: It is a new approach to enhance the permeability of liposomes by adding a high concentration of glycerol (10%–30%, v/v) and named the novel vesicular preparations as "glycerosomes (Zhang et al, 2017). Glycerosomes are prepared by hydrating dipalmitoylglycerophosphatidylcholine-cholesterol films with glycerol aqueous solutions (10–30%, v/v) (Maria et al, 2013). In many works, glycerosomes are prepared and tested as skin delivery systems of natural and synthetic compounds (Maria et al. 2015).

.(Shankar et al, 2018).

Glycethosomes: It is a modified liposomal vesicle composed of a combination of glycerol-ethanol and phospholipid vesicles (glycethosomes). The analysis of research studies demonstrated that each molecule requires a specific adhoc tailored phospholipid vesicle formulation to maximize the efficacy. (M. Pleguezuelos-Villa et al. 2019).

Proniosomes

Proniosomes are dry formulation of water soluble carrier particles that are coated with surfactant. They are rehydrated to form niosomal dispersion immediately before use on agitation in hot aqueous media within minutes. Proniosomes are physically stable during storage and transport. Drug encapsulated in vesicular structure of proniosomes prolong the existence of drug in the systematic circulation and enhances the penetration into target tissue and reduce toxicity. (Radha et al. 2013).

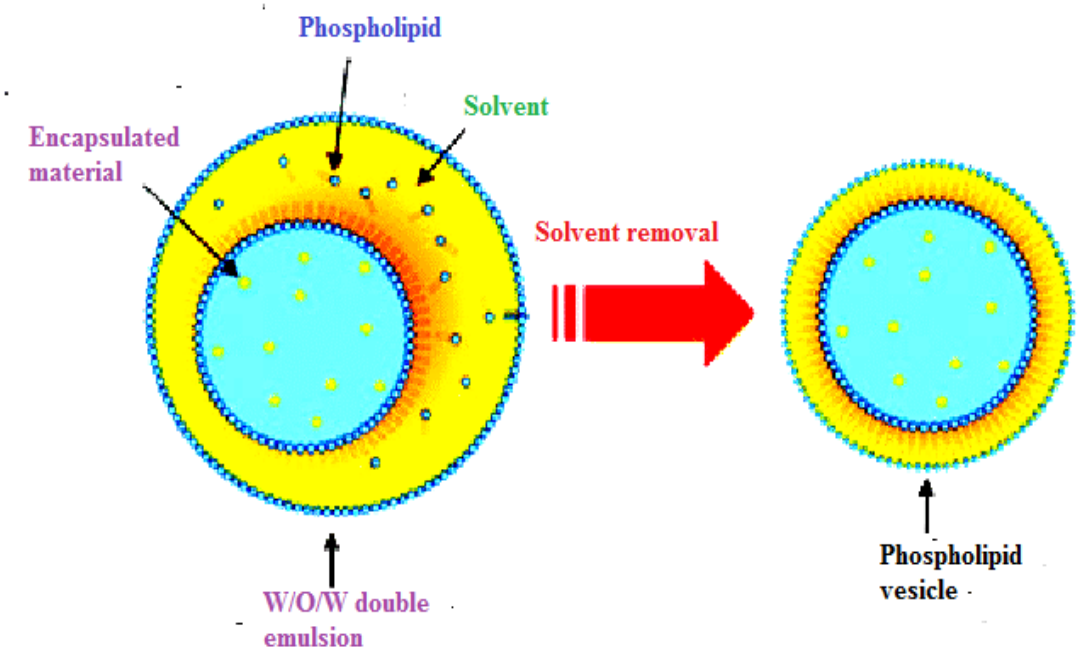

Fig 2. Phospholipid Vesicles

Merits of nanovesicles over conventional dosage forms

- Phospholipid vesicles have a great ability to enhance the dermal and transdermal delivery of many drugs so preventing the drug degradation in gastrointestinal tract and the drug metabolism in liver (Heba et al, 2018).
- They have high diffusion rate of the drugs across the layer of skin (Shankar et al, 2018).
- They have higher bioavailability and much lesser side effects (M. Pleguezuelos-Villa et al, 2019).
- The nanovesicle system is passive and non-invasive.
- The dose requirement is reduced due to improved absorption of the main constituent. They can also be given in smaller quantities to achieve the desired results.

Drug profile (berberine)

Natural products are valuable sources in novel drug development. Berberine is a yellow alkaloid which occurs in numerous plants. It was first isolated from *Xanthoxylon cava* in 1826 by Chevallier and Pelletan. Berberine is an isoquinoline derivative alkaloid isolated from many medicinal herbs, such as *Hydrastiscanadensis* (goldenseal), *Cortex phellodendri* (Huangbai), *Rhizomacoptidis*(Huanglian) Berberisaristata, *Coptischinensis, Coptis rhizome, Coptis japonica, Phellondendronamurense*and *Phellondendronchinenseschneid* (Imanshahidi and Hosseinzadeh, 2008). The diverse pharmacological properties exhibited by berberine indicate that the alkaloid has a definite potential as drug in a wide spectrum of clinical applications. In this respect, the structure of berberine (Fig. 3) represents a biologically important skeleton and also an attractive natural lead compound for the introduction of various chemical modifications in appropriate positions, in search for more selective, discriminated and narrowed medical indications (Kumar et at, 2015). In Chinese pharmacopoeia, Huangbai and Huanglian are described as 'heat-removing' agents for their fever reducing therapeutic application.

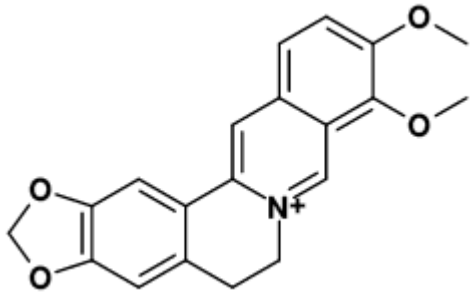

Fig 3. Structure of berberine

Clinical uses of berberine

Berberine is an isoquinoline alkaloid with many therapeutic effects including anti-viral, anti-microbial, anti-diarrhea, **anti-psoriatic, anti-inflammatory** and anti-tumor effects(Nimisha et al, 2020; Kulkarni and Dhir, 2010). Berberine has some significant effects on type 2 diabetes through adenosine monophosphate-activated protein kinase activation, glycolysis stimulation, and mitochondrial function inhibition which subsequently improves both lipid and glucose metabolism. Some other effects of berberine on congestive heart failure, cardiac

arrhythmia, antibiotic, wound healing, antisoriatic and hypertension have been reported (Caliceti, et al, 2016; Vuddanda et al, 2010; Bhatia *et* al., 2021; Zhou et al., 2021; Sondhi et al., 2021, Vanti et al., 2021). **Beside the beneficial effects of berberine, some limitations including poor aqueous solubility, slight absorption, and low bioavailability have hindered its applications**. **To overcome these limitations, nanotechnology has been considered as main strategy. Therefore, the aim of the present work is to develop a nanovesicles formulation of berberine and study its effects in an IMQ induced psoriasis model as well as carrageenan induced inflammation model.**

Literature Review

- Zhou et al. (2021) proved that berberine hydrochloride has good wound healing effect
- Sondhi et al. (2021) demonstrated that topic gel of berberine hydrochloride has strong anti-psoriasis effect
- Nimisha et al. (2020) developed an Antipsoriatic and Anti-inflammatory Studies of Berberisaristata Extract Loaded Nanovesicular Gels and conculed that topical application of extract loaded transferosomal gel showed a marked reduction in thickness of epidermis, length of rete ridges as compared to conventional gel formulation. It can be inferred that B. aristata extract loaded transferosomal gel can function as potential anti-inflammatory and antipsoriatic formulation.
- M. Pleguezuelos-Villa et al. (2019) developed a Mangiferin glycethosomes as a new potential adjuvant for the treatment of psoriasis and concluded that mangiferin loaded glycethosomes respect to the mangiferin dispersion to promote the heal of the wound induced by TPA, confirming their potential application for the treatment of psoriasis or other skin disorders.
- Shankar et al. (2018) developed a topical delivery of drugs using ethosomes: A review and concluded that Topical drug delivery system is a system where the drug reaches the systemic circulation through the protective layer i.e. skin. The main disadvantage of this route is the low diffusion rate of the drugs across the layer of skin which is the stratum corneum. To overcome this problem to a certain extent, ethosomal delivery for drugs and herbal compounds has been recently introduced.
- Zhang et al. (2017) developed an essential oil-mediated glycerosomes increase transdermal paeoniflorin delivery and concluded that The glycerosomes mediated by STO exhibited considerable skin permeability as well as improved drug absorption in the synovium, indicating that STO-glycerosomes may be a potential PF transdermal delivery vehicle for the treatment of rheumatoid arthritis caused by synovium lesions.
- Maria et al. (2017) developed a Glycerosomes: investigation of role of 1,2-dimyristoyl-sn-glycero-3 phosphatidycholine (DMPC) on the assembling and skin delivery performance

and concluded that . The addition of glycerol led to the formation of more viscous systems (from ~2.5 mPa/s for basic liposomes to ~5 mPa/s for glycerosomes), which improved spreadability of the formulations on the skin. Results obtained in vitro were promising using glycerosomes.

- Benesi et al. (2016) developed anti-Psoriasis Agents from natural plant sources and conclude that psoriasis is a chronic inflammatory immune-mediated skin disease. It affects most races, does not have any sexual predilections and can manifest at any age of life. Psoriasis is more frequent in certain racial groups and geographical areas. The research on natural products has greatly contributed to the progress in the treatment of skin diseases such as psoriasis and many of these compounds are now being used. Understanding the mechanism of these molecules will contribute to the development of more specific preventive strategies for the treatment of psoriasis.
- Virginia et al. (2016) developed a inhalable polymer-glycerosomes as safe and effective carriers for rifampicin and concluded that the improvement of rifampicin accumulation in lungs
- Maria et al. (2015) developed a nanoincorporation of curcumin in polymer-glycerosomes and evaluation of their in vitro-in vivo suitability as pulmonary delivery System and concluded that the curcumin accumulation in the lungs confirming their potential use as a target system for the treatment of pulmonary diseases.
- Gregory et al. (2012) developed a liposomes in drug delivery and concluded that liposomes (phospholipid-based vesicles) used as particularly in areas such as cancer chemotherapy, antimicrobial therapy, vaccines, diagnostic imaging and the treatment of ophthalmic disorders has indicated clearly that some liposome-entrapped drugs and vaccines exhibit superior pharmacological properties to those observed with conventional formulations.

Aims and objectives of the study

Due to poor aqueous solubility, slight absorption, and low bioavailability of berberine hinder its applications. To overcome these limitations, phyto nanotechnology has been considered as main strategy. Therefore, the aim of the present work is to develop a nanovesicle formulation of berberine. The main objective of this research work is to formulate a novel flexible vesicular formulation loaded with berberine then formulated these vesicles as topical formulation to enhance the solubility, the bioavailability and avoid the systemic serious adverse effects. The studies will be carried out with the following objectives:

1. To prepare the berberine Chloride loaded nanovesicles carrier for better effect.
2. To characterization of the formulation
3. To check the anti-psoriatic and anti-inflammatory effect on animal model.

Proposed Research Methodology

1. Collection and authentication of selected drug, surfactant and penetration enhancer:

The ingredients of selected formulation will be collected from appropriate sources.

1. **Preparation of the vesicles:** Berberine Chloride loaded nanovesicles will be prepared.
2. **Characterization of vesicles**

A) Morphology and particle size: Transmission Electron Microscopy (TEM) can be used to determine particle size distribution and surface morphology of the complex.

B) Diameter and polydispersity index (PI): Photon Correlation Spectroscopy (PCS) will be used for determination of diameter and polydispersity index.

C) Zeta Potential: Zetasizernano-ZS will be used for determination of zeta potential.

D) Entrapment efficiency (EE): The entrapment efficiency of a phytosomal formulation can be determined by subjecting the formulation to ultracentrifugation technique.

E) In vitro diffusion studies: Release characteristics will be determined as per the dialysis method.

A) Stability studies: The drug retention behavior of vesicles will be evaluated by storing the nanovesicle formulations at a refrigeration temperature (2–8 °C) throughout the study.

B) Solubility Studies: Solubility of the drug, phospholipids, their physical mixture and the nanovesicle formulation can be evaluated.

3. Biological activity (anti-psoriatic and anti-inflammatory)

In vivo studies

For evaluation of anti-psoriatic activity

- IMQ cream can be used to induce Psoriasis in animal model
- Five‑week‑old male or female mice with no previous drug treatment will be used for *in vivo* studies
- Antipsoriasis activity can be evaluated by histopathological study

For evaluation of anti-inflammatory activity

- Carrageenan can be used to induce inflammation
- Wistar rats of either sex (150–200 g) with no previous drug treatment

  Can be used for *in vivo* studies.
- Anti-inflammatory activity can be evaluated by measurement of Carrageenan‑induced paw edema.

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